

Title: CHIMERIC, HUMAN AND
HUMANIZED ANTI-GRANULOCYTE
ANTIBODIES AND METHODS OF USE
Inventor(s): GOLDENBERG et al.
Atty. Dkt. No.: 018733-1267

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AGCATTGTCGATGACGACAGCTCCCTCCCTGTCTGTCGAGATCAGCCTCCATCTCTTCGAGATCTAGTCAGACGATTGTA 90
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TCGTAACACTACTGGCTCTGAGGTGAGGGGAGGACAGCAGTCAAGAACTCTAGTTGCGAGGTAGAGACGCTTAGATCAGTCTCGTAACAT 27A B C
1 10 20 30
S I V M T Q T P L S L P V S L G D Q A S I S C R S S Q S I V
CATACTAATGACAAACCTATTAGATGGTACTTCGAGAACCGCCAGCTCTCAACCTCTCATCTACAGAGTTTCCACCGATT 180
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
GTATCTTACTTTGTGATAAATCTTACCATGGAGCTCTTTGGTCGGGTGACAGGTTTGAGGAGTAGATGTTTCAAGGTTGGCTAAA 50
D E 30 40 50
H S N G N T Y L E W Y L Q K P G Q S P N L L I Y K V S N R F
CDR1
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
TCGCGGTCCTCCACACAGGTTTCAGTGGAGTGGATCAGGACAGATTTCACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGAGTT 270
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
AGACCCGAGGCTCTGTCCAAAGTCACGTCACCTAGTCCCTGTCTAAAGTGTGAGTCTTAGTGTCTCACCTCCGACTCTTAGACCTCAA 80
S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V
TATTACTGCTTTCAAGGTTCAATGTTCTCCAGCTTTCCGGTGAGGACCAAGCTGGAAATCAACCGGctgatgctgcaccaactyta 339
ATATGACGAAGGTTCCAAGTGTACAGGAGGCTGCAAGCCACCTCCGTGGTTTCGACCTTTAGTTTGGCCgactacgacgtgggtgacat
90 100 108
Y Y C F Q G S H V P P T F F G G G T K L E I K R
CDR3
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
tcaatcttccacacatccagtgaagatccggc
-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+
aggtagaagggtggtaggtcaactcctctaggccg

```

Figure 1A shows the DNA sequence encoding MN3Vκ cloned by RT-PCR and the predicted amino acid sequence. Underlined arrows indicate the PCR primer sequences. The putative CDR regions are in bold and underlined, and indicated. Nucleotide residues are numbered sequentially (right side). Kabat's Ig molecule numbering is used for amino acid residues (top of the residues).

```

90
CAGTCCACTGCGAGAGCTGGACCTGAGAGAGCTGGAGAGAGCTGAGAGATCCTGGAGAGCTTCGGGTATACCTTCAGA
-----
GTCAGGTTGAGCTGCTCAGAGCTGGAGCTGACCTCTTCGGAGCCTCTCTGAGTTCTATGAGAGCTTCGGAGAGCAGCATATGGAAGTCT
-----
1 10 20 30
Q V Q L Q E S G P E L K K P G E T V K I S C K A S G Y T F R
-----
180
AACTATGGAATGATCTGGGTGAAACAGCTCCAGGAAAGGGTTTAAAGTGAATGGCTGGATAAACACCTACCTGGAGAGCAACATAT
-----
TTGATACCTTACTTGAACCACTTTGTCGAGCTCTTTCCCAATTTCACTACCGAGCTATTCTGTGAGTGTGACCTCTCGGTTGTATA
-----
1 50 52A
N Y G M N W V K Q A P G K G L K W M G W I N T Y T G E P T Y
-----
CDR2
-----
270
GCTGATGACTTCAGGAGCGTTTGCTTCTTTGGAACTCTCCAGCACTGCTCTATTGGAGATCAACACGTCACAAATGAGGAC
-----
CGACTACTGAGCTTCCTCGCAACGGAGAGAACCTTTGGAGAGCGTGTGACGGTAAACGCTCTAGTTGTCGAGTCTTTTACTCCTG
-----
60 70 80 82A B C
A D D F K G R F A F S L E T S A S T A Y L Q I N N V K N E D
-----
360
AGGCTCATATTTCTGTGCGAAGAGGATGAGATTTCAACGGTAGTAGCTCGACTACTGGGGCCAGGAGACCGGTACCGTC
-----
TGCCGATGATAAAGACACGTTCTTCCCTACTACCTAAAGTTGCCATCATCGAGCTGATGACCGGTTTCCTGTGTCACGTGGCAG
-----
90 100 A B C D E 110
T A T Y F C A R K G W M D F N G S S L D Y W G Q G T T V T V
-----
CDR3
-----
366
TCCTCA
-----
AGGAGT
-----
113
S S

```

Figure 1B shows the DNA sequence encoding MN3VH cloned by RT-PCR and the predicted amino acid sequence. Underlined arrows indicate the PCR primer sequences. The putative CDR regions are in bold and underlined, and indicated. Nucleotide residues are numbered sequentially (right side). Kabat's Ig molecule numbering is used for amino acid residues (top of the residues).

```

GACATCCAGCTGACCCAGACCTCCACTCTCCCTGCTCAGTCTTGGAGATCAAGCTCCATCTCTTGAGATCTAGTCAGAGCATGTA
90
CTGTAGGTCGACTGGGTCTGAGGTGAGGGGAGGAGCAGTCAGAACCTCTAGTTGGAGGTAGAGAAGCTCTAGATCAGTCTCGTAACAT
30
D I Q L T Q T P L S L P V S L G D Q A S I S C R S S Q S I V
CATAGTATGGAACACCTATTAGAAATGGTACCTGCAGAACACAGGCCAGTCTCCAAACCTCCTCATCTACAAAGTTTCCACGATTT
180
GTATCATACCTTTTGGATTAATCTTACCATGGACGCTCTTGGTCGGGTGAGAGGTTTGGAGGAGTAGATGTTTCAAGGTTGCGTAA
H S N G N T Y L E W Y L Q K P G Q S P N L L I Y K V S N R F
55
CDR1
TCTGGGTCCTCCAGACAGAGTTCAGTGGCAGTGGATCAGGGACAGATTTACACTCAAGATCAGACAGAGTGGAGGCTGAGGATCTGGAGTT
270
AGACCCGAGGGTCTGTCCAAAGTCACCGTCACCTAGTCCCTGCTTAAGGTGTAGTTCTAGTCGTCTCACCTCGGACTCCTAGACCCCTCAA
S G V P D R F S G S G T D F T L K I S R V E A E D L G V
85
TATTACTGTTTTCAAGGTTTCACATGTTCTCCACAGTTCGGTGGAGGACCAAGCTGGAGATCAAAAGT
339
ATATGACGAAAGTTCCAAAGGTACAAAGGAGGCTGCAAGCCACCTCGGTGGTTCGAGCTCTAGTTTGA
Y Y C F Q G S H V P P T F F G G G T K L E I K R
108
CDR3

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Figure 2A shows the DNA and amino acid sequences of cMN3Yk domain. The CDR regions are in bold, underlined, and indicated. Nucleotide residues are numbered sequentially. Kabat's Ig molecule numbering is used for amino acid residues (same as in Fig. 1A).

CAGGTCACACTGCGAGGAGTCTGGACCTGAGCTGAGAGAGCTGGAGAGACAGTCAGATATCTCTGAGGCTCTGSGTATCTACTTCAGA
 90
 GTCCAGGTTGACGTCTCAGACCTGACTGACTTCTTGGACCTCTCTGTCAGTTCTATAGAGGCTTCCGAGAGACCCATATGGAAGTCT

 Q V Q L Q E S G P E L K K P G E T V K I S C K A S G Y T F R 30

 AACATGAGATGAAGTCTGGAGGAGCTTCCAGGAGGAGGTTTAAAGTGGATGGCTGGATGACACCTACACTGGAGAGCCACATAT
 180
 TTGATACCTTACTTGGACCCACTTGTCCGAGGCTCTTCCCAAAATTCACCTACCGAGCTATTTGTGGATGCGACCTCTCGGTTGTATA

 N Y G M N W V K Q A P G K G L K W M G W I N T Y T G E P T Y 59
 CDR1

 GCTGATGACTTCAGGAGGAGGTTTGCTCTCTTGGNAGCTCTGCCAGCACTGCTCTATTTGCGATCAGACAGCTCAAAATGAGGAC
 270
 CGACTCTGAGGTTCCCTGCCAAGCGAGAGAGAACCTTTGGAGAGGCTGCTGACGGATAAAGCTCTAGTTGTTGACGTTTTTACTCTCTG

 A D D F K G R F A F S L E T S A S T A Y L Q I N N V K N E D 86

 ACGGCTACATATTTCTGTGGAAGAGGAGTGGATGGATTTCAAGGATGAGCTGACTGAGCTGGGGCCAGAGGACAGGTCACCGTC
 360
 TGCCGATGATTAAGACACAGGTCTTTCCCTACTACTACTAAGTTTCCATCATCTGGAGCTGATGACCCCGGTTCCCTGCTGCCAGTGGCAG

 T A T Y F C A R K G W M D F N G S S L D Y W G Q G T T V T V 111
 CDR3

 TCCTCA 366
 ACGAGT
 S S 113

Figure 2B shows the DNA and amino acid sequences of cMN3VH domain. The CDR regions are in bold, underlined, and indicated. Nucleotide residues are numbered sequentially. Kabat's Ig molecule numbering is used for amino acid residues (same as in Fig. 1B).

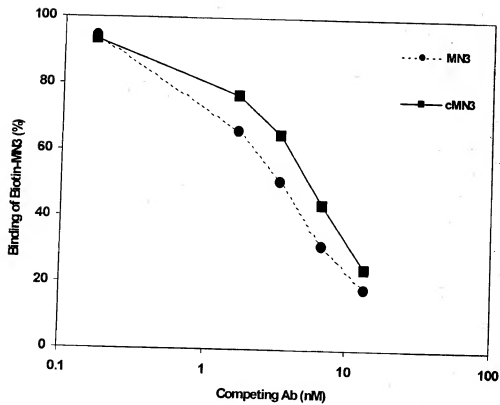


Figure 3.

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2

9

Figure 4A. Amino acid sequence alignment of REI, MN3 and hMN3 light chain variable domains. Dots indicate the residues in MN3 is identical to the corresponding residues in REI. Dashes represent gaps introduced to aid the alignment. Boxed represent the CDR regions. Both N- and C-terminal residues (underlined) of hMN3 are fixed by the staging vector used. Therefore, the corresponding terminal residues of MN3 are not compared with that of REI. Kabat's Ig molecule numbering scheme is used (same as in Fig. 1A).

XbaI
tctagacacagagacctaccatGGGATGGAGCTGTATCATCTCTCTTTGGTAGCAGACGTACaggtta
M G W S C I I L F L V A T A T

aggggctcacagtagcagggcttgaggtctgacatatatgggtgacaatgacatccacttgcctttctctccacAGGTGTCCACTCC
G V H S

PvuII
GAGATCCAGCTGACCCAGAGCCCGACGAGCTGAGCGCCAGCGTGGGTGACAGAGTGTCCATCTCTGTGTAGATCCAGTCAGACGATTGTA 90
D I Q L T Q S P S L S A S V G D R V S I S C R S S Q S I V 27C

CDR1
CATAGTAATGAACAACCTATTAGATGGTACCAGCAGAGCCAGGTAGGCTCCAAAGCTGCTGATCTACAAAGTTTCCACCGATT 180
H S N G N T Y L E W Y Q Q K P G K A P K L L I Y K V S N R F 55

CDR2
TCCGAGTGCCAGACAGATTACGCGGTAGCGGTAGCGACTTCACCTTCACCATCAGACGCTCCAGCCAGAGGAGCATGCCACC 270
S G V P D R F S G S G T D F T F T I S S L Q P E D I A T 85

BelII/BclI
TACTACTGCTTCAGGTTTCATGTCTCCAGCTTCGGCGCGGACCAAGGTGGAGATCAGAGtgagtagaatttaaactttgct 337
Y I C F Q G S H V P F T F G G G T K V E I K 107

BamHI
tctcagttggtacc

CDR3

Figure 5A.

XhoI
ctcgaagcacacaggacctcaccatgggagctgattatcctctcttcttgtagcagctacaggtla
M G W S C I I L F L V A T A T
aggggctcacagttagcaggcttgaggtctcggaacatatatagggtgacaatgacatcacctttgctctctctccacagctgtccactcc
G V H S
PstI
CAGGTCCAACTGCAGCAGCTCTGAGCTGAGGTCAAGAGCGCTGAGCTAGCTCTCCTGCAAGGCTTCGGGTATACCTTCAGA 90
Q V Q L Q S G A E V K K P G S S V K V S C K A S G Y T F R 30
TACTATGGAATGACTGGGTGAGACAGCGCTCCAGCAGCGTTTACAGTGGATGGCTGATPAACACCTACACCGGTGAGCCACATAT 180
N Y G M N W V R Q A F G Q G L E W H G W I N I Y T G E P T I 59
CDR1
GCTATGACTTCAGGGAGCGTTTCCTTCACAGCCGACGAACTACCAACACTGCTATATATGAGAGCTGTCTAGCTTCAGATCTGAGGAC 270
A D D F K G R F A F T A D E S T N T A Y M E L S L R S E D 75
ACGGCTTCTATTCTGTCGACGAAAGGATGGAGTTCACGGTACCTGACTACTGGGCGCAAGGAGCCCGGTCTACCTC 360
I A F Y F C A R K G W M D F N G S L D I W G Q G T P V T V 111
CDR3
TCCTCAGTgagctcttaacacctctctctctattcagcttaaatagattttactgcatttgggggggaaatggtgtatctgaat 367
S S
ttcaggctcagaaggactagggacacctggggagtcagaagggtcattggggagccgggctgatgcagacagacatcctcagctccag
BamHI
acttcattggccagagatttataggatcc

Figure 5B.

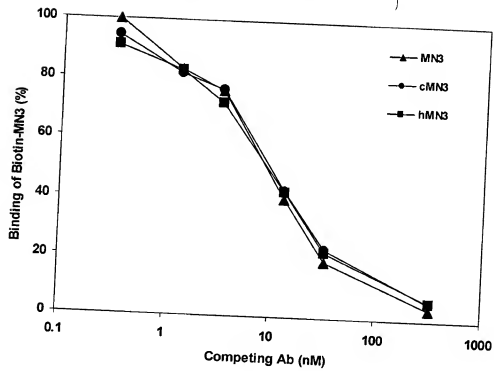


Figure 6.